OnlineMedEd

# Innate Immune Response

## Intro

Immunity starts and ends with the phagocytes of the innate immune system. There are seven lectures about what happens in between—in particular, the involvement of the adaptive immune system—but all of that just helps and enhances what is already present in the innate immune system. This lesson focuses on the action of phagocytic cells (particularly neutrophils and macrophages).

Seen from 30,000 feet, the idea is that the cells already present in the tissue being invaded do what they do (phagocytosis), and when they do that to a foreign antigen, they call for help. The **local event** is the original phagocytosis of a foreign antigen, mediated by very general mechanisms. The phagocytes phagocytose one of the antigens, then call for help by releasing cytokines. These cytokines induce **vascular events** in the local tissue, resulting in vasodilation to bring more blood to the area being invaded and causing pooling on the venous side. These vascular events allow for the **cellular events** by which circulating monocytes are able to slow down, get out of the capillaries and into the tissue, and help fight the antigen. Eventually, a very small number of those monocytes-turned-macrophages will be dispatched to a secondary lymphoid organ, which bridges the innate immune system and the adaptive immune system. The adaptive immune system then either releases antibodies to facilitate phagocytosis, showing the innate immune system what to phagocytose, or releases cytokines to kill the invader and/or supercharge the phagocytes.

Remember that a macrophage is a monocyte-in-the-tissue. So we'll use "macrophage" generally to mean both monocyte-in-the-blood and monocyte-in-the-tissue.

I use the analogy of a war zone. There are forward scouts who monitor for enemy activity (neutrophils). When they spot an enemy, they raise the alarm (cytokines). This alerts the shock troops in the nearby barracks (macrophages), who are better at fighting than the scouts, but who can't always be on the front lines. They're ready to respond, and when the alarm is raised, they deploy in force and number. They know where to go because they have the scouts' signal (cytokines induce vascular events), and are dispatched onto the front lines because the message (cytokines) gets louder when they are going in the right direction (the source of cytokine release). Eventually, some of those macrophages will take information back to the forward command post (secondary lymphoid organs), where the information will be processed by the officers there (lymphocytes), and specific units (T cells) and specialized weaponry (antibodies) trained to fight the invader will be dispatched to further bolster the front-line soldiers.
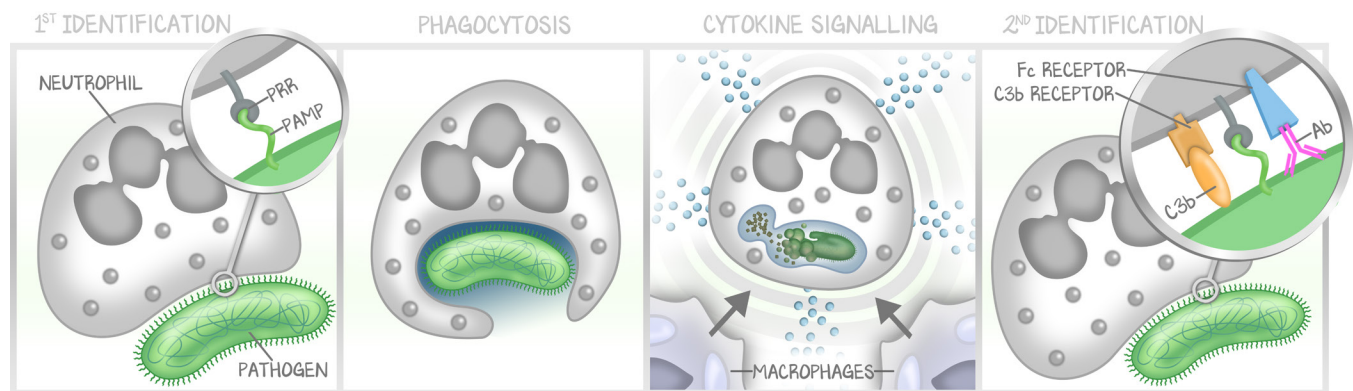
This lesson is about everything up to the macrophage leaving for the secondary lymphoid organ. Of course, it's more complicated than we make it. But if you learn it as neutrophils–scouts, macrophages–shock troops, it will be much easier to understand. And it follows the pattern of wound healing: neutrophils first, macrophages to finish it off, like what you learned in Inflammation and Neoplasia #4: *Wound Healing*.

| | CELLS/EVENTS | ROLE IN THE IMMUNE RESPONSE |
|---|---|---|
| **LOCAL EVENTS** | Phagocytosis cytokines | Initial tissue invasion by a pathogen and the response of the front-line border patrol. |
| **VASCULAR EVENTS** | Arterial vasodilation: ↓ resistance = ↑ into area<br>Venous vasodilation: ↑ permeability, ↑ stasis | Means by which the flow is slowed and the capillaries opened. |
| **CELLULAR EVENTS** | Margination emigration<br>• Rolling, activation (IL-8), adhesion, migration chemotaxis<br>Phagocytosis | Circulating leukocytes getting out of the blood, into the tissue, and fighting the invaders. |

**Table 4.1: Events of Innate Immunity**

## Local Events

Those forward scouts, those phagocytic neutrophils, are there looking for enemies. When they find one, they send the call for help by releasing cytokines. Cytokines cause the vascular changes locally that bring the backup shock troops. But how does the neutrophil know it's found an enemy? How does a neutrophil know what it should phagocytose? The innate immune system is **general** and **lacks memory**. That means the mechanisms by which an antigen is identified must be **generic**. The details of toll-like receptors and memorizing the litany of types are irrelevant for medical immunology. Just know that a **pattern-recognition receptor** (PRR) on the phagocyte is activated by **pathogen-associated molecular patterns** (PAMPs). When the phagocyte bumps into another thing with a PAMP, its PRR links up, and the process of phagocytosis is started. That process also releases the cytokines that initiate the vascular events.



**Figure 4.1: PRRs and PAMPs**
A phagocyte knows to engulf, ingest, and digest a pathogen because the phagocyte has a nonspecific PRR (a receptor) that attaches to a PAMP on the pathogen. The initiation of phagocytosis also releases cytokines. The innate immune system has a limited arsenal of enemy-recognition tools: just PRRs. Complement (also part of the innate immune system) can facilitate phagocytosis. The adaptive immune system provides help to the phagocyte through several tools; supercharged cytokines (death cytokines to the pathogen), as well as antibodies that opsonize the pathogen.

## Vascular Events

Because of physics (laminar flow), a circulating leukocyte is confined to the middle of a capillary when blood is flowing. This is the default. At the middle of the artery, leukocytes are flowing quickly, and can't slow down, hold on, or exit. The local events where an enemy invader is being attacked result in the release of cytokines. Wherever cytokines are released is where the vascular events will occur. At the site of the **arterioles**, **vasodilation** reduces the vascular resistance, resulting in more blood flow into that location. Leukocytes are measured in cells/mL. Therefore, bringing more blood locally, that is, bringing more mL locally, means that more leukocytes are brought to this region locally. On the **venule** side, vasodilation causes stasis and increased vascular permeability. **Stasis** allows escape from laminar flow, permitting circulating leukocytes to drift to the edge of the capillary where they can slow down and grab hold. **Increased vascular permeability** lets leukocytes out of the capillary into the local tissue.

To summarize, the idea is that these circulating leukocytes are floating around all the time. They're moving too fast to slow down. They're too far away from the endothelium to try to leave, and even if they could get closer, the capillary walls are too impermeable for them to get out. But when there's a signal that help is needed, they slow down, migrate to the capillary wall, and then loose capillaries let them out. When you injure your knee, the trauma leads to vascular events that cause your knee to get hot (inflammation and vasodilation) and swollen (permeability). The symptoms are actually a response to trauma. The response allows the immune cells get to the area of injury to repair injury and fight infection.

## Cellular Events

There are four events that brings a cell out of the bloodstream to the tissues where they can do the work (killin' bad guys) of the innate immune system: margination, emigration, chemotaxis, and phagocytosis. Stasis reduces laminar flow, allowing circulating leukocytes to move from the center of the lumen to the edge (a process called **margination**). This brings the leukocytes near the site of invasion. Cytokines help the leukocytes become "sticky" so they can grab onto the vascular wall. **Emigration** is the process by which the slowed leukocyte stops at the site of invasion and leaves the capillary, into the tissue. This is a complex process which varies according to how strongly the leukocyte sticks to the edge of the capillary walls and how much vascular permeability is increased. Once in the tissue, the leukocyte has to get from the capillary wall to the exact location where inflammation (or the "fight") is, a process called **chemotaxis**. When at the site of the pathogen, the leukocyte kills that pathogen, usually through **phagocytosis**. We explore each in step in detail below.

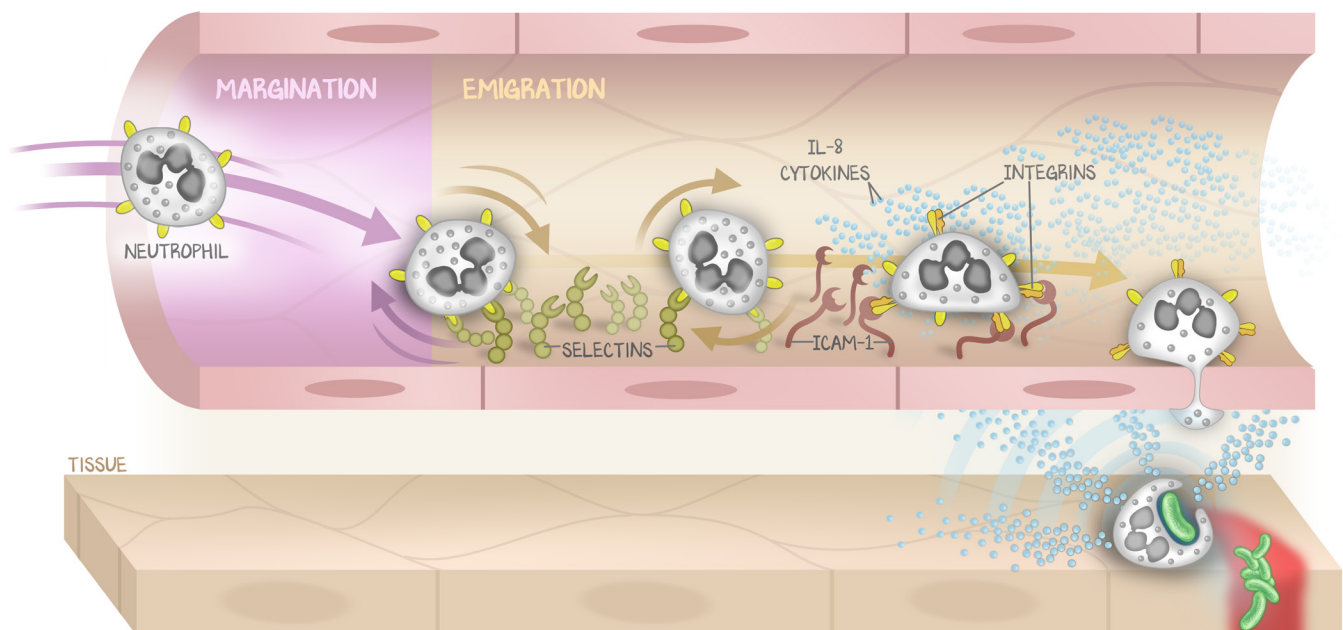| PHASE | ACTIVITY |
|---|---|
| Margination | Vascular events, vasodilation, laminar flow |
| Emigration | Rolling (selectins) <br> Activation (cytokines from local leukocytes) <br> Adhesion (integrins) <br> Migration (podocytes) |
| Chemotaxis | Chemoattraction (cytokines from local leukocytes) |
| Phagocytosis | Attachment <br> Ingestion <br> Bacteriolysis <br> Expulsion |

**Table 4.2**
The four phases of phagocytes leaving the blood, entering tissue, and engulfing bacteria.

**Margination** occurs as stasis develops in the local area. The axial stream of free-flowing arterioles confines large cells to the center of the lumen. At the site of injury, that axial stream is lost as vasodilation causes stasis, allowing leukocytes to reach the edge of the vessel wall. The leukocytes "marginate" towards the capillary wall. Margination is just the process of physics allowing the cells to leave the center of high laminar flow and reach the edge of the capillary, so they can grab onto the vessel wall and begin emigration.

**Emigration** is subdivided into four phases: rolling, activation, adhesion, migration. Emigration begins when margination finishes (the blood flow slowed down enough so the cell can stop) and ends when the leukocyte has entered tissue. Leukocyte **rolling** is what the leukocytes are already doing just by the physics of having been brought in by blood flow. Even though there has been sufficient stasis to allow margination, they are still going too fast to simply stop on their own. As they roll over the capillary walls, there are little handrails that the leukocyte grabs onto and tries to stop. These handrails are called **selectins** and allow the leukocyte to **stick** to the capillary wall. This can happen anywhere, but it isn't strong enough to stop the leukocyte; it just slows it down more. When a leukocyte gets microscopically close to the site of injury, cytokines (**interleukin 8**) from local defenders **activate** the circulating leukocyte to express integrins, which have a stronger hold than selectins. This makes the cells stickier at the site of invasion. Selectins are always expressed, and are weak. Integrins are turned on by cytokines and are strong. **Integrins** allow for **invasion** by binding **ICAM-1**. Through the combination of the selectins slowing and the integrins braking, the circulating leukocyte stops (arrests). The arrested (stopped) circulating leukocyte

then uses podocytes to **migrate** across the capillary endothelial lining, squeezing between two capillary endothelial cells which are already spaced apart as a result of vasodilation.

**Chemotaxis** is the process by which the phagocyte is directed to the exact spot of the bad guys. The initial neutrophils (the first to arrive) or the macrophages in the tissue release chemoattractants. These chemoattractants allow other phagocytes to follow the trail, working their way up the concentration gradient towards the source of the chemoattractant—from the capillary wall to exactly where the pathogen is. The concentration of the chemoattractant gets higher closer to the source of its release, analogous to a siren that sounds louder as you get closer to it.



**Figure 4.2: Cellular Events**
An illustration of each cellular event as time passes, left to right, resulting ultimately in a phagocyte at the site of the pathogen.

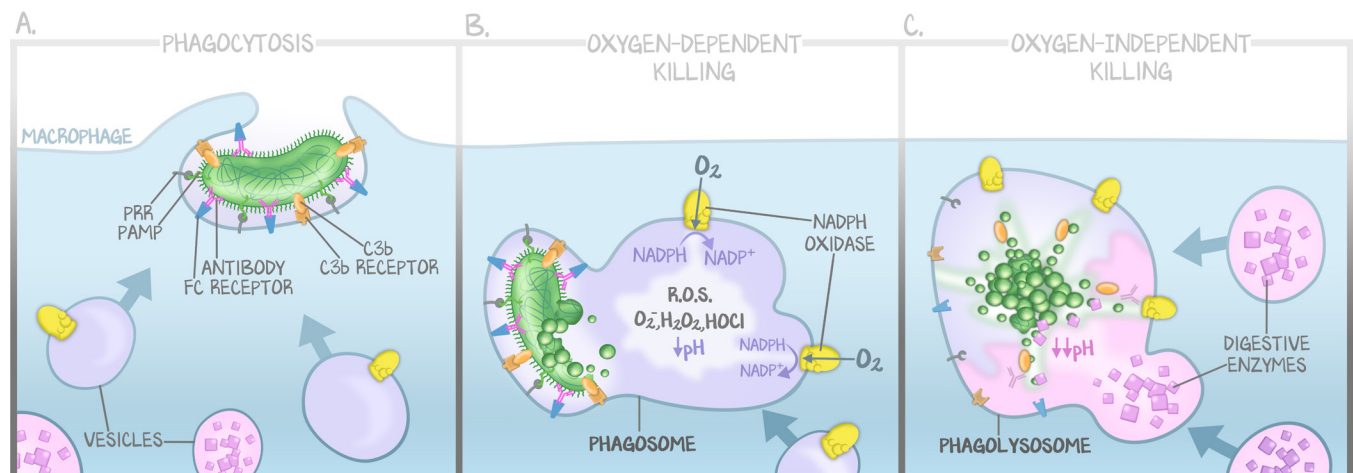**Phagocytosis** is the final step in cellular events, but is involved enough to deserve its own section.

Vascular events bring the cells to the right organ. Cellular emigration brings cells to the right capillary. Cellular chemotaxis brings cells to the right spot. Cellular phagocytosis kills the pathogen.

## Phagocytosis

Phagocytosis is the cornerstone of cellular defense. Phagocytosis is the impetus by which "local events" occur and then start the entire process of the innate immune system. Phagocytosis also allows some of these innate immune cells to present antigens to T cells in secondary lymphoid tissues. T cells bridge the innate and adaptive immune systems, dispatching immunoglobulin-producing B cells and cytotoxic T cells. Antibodies come full circle and connect the innate and adaptive immune systems. Immunity starts with the innate immune system, with phagocytosis. The adaptive immune system supercharges phagocytosis via T cells and antibodies. The antibodies bind to foreign invaders they recognize and make it easier for those pathogens to be phagocytosed by the cells of the innate immune system. The innate immune system will then present the foreign invader to the adaptive immune system and activate B cells that become antibody-producing plasma cells. Then the antibodies produced by the plasma cells circulate to help the innate immune system attack a future foreign invader. So you can see how the innate and adaptive immune systems are continuously interconnected.

Phagocytosis progresses through attachment, ingestion, bacteriolysis, and then exocytosis of particles. Phagocytosis begins with **attachment**. Phagocytic cells possess receptors called **pattern-recognition receptors** (PRRs) that recognize antigens (molecular patterns) on bad cells. These bad cells could be foreign pathogens which intrinsically produce nonspecific **pathogen-associated molecular patterns** (PAMPs). These bad cells could also be dying or damaged self-cells that need to be cleared out, and produce **damage-associated molecular patterns** (DAMPs) that help them to be removed. These PRR receptors are innate and intrinsic, which means they aren't very specific. The rate of attachment is increased with **opsonization** by antibodies (#6: *Antigens and Antibodies*) and reduced by bacterial pili and capsules that resist phagocytosis. PRRs are also known as toll-like receptors (TLRs). Once attached, the phagocyte **ingests** the foreign particle. Ingestion begins by evagination, in which the cell extends its plasma membrane around the antigen. Once the plasma membrane is closed, the particle is now enclosed in a **phagosome**.

The cell has already made several **lysosomes**, each one a storehouse of anti-pathogen weaponry. Lysosomes are filled with granules. The phagosome and lysosome fuse together, releasing the granules from the lysosome into the same space as the stuff in the phagosome. This process of releasing granules is called degranulation. **Bacteriolysis** is achieved through the fusion of the killer-content-containing lysosome and the enemy-containing phagosome and degranulation. The granules do their thing, the pathogen is destroyed (bacteriolysis), and then the phagocyte kicks out the destroyed bacteria particles (exocytosis of particles).



**Figure 4.3: Oxygen-Dependent and Oxygen-Independent Intracellular Digestion**
(a) Phagocytosis is initiated by PAMP-PRR activation and enhanced by activation of Fc-receptor and complement activation. (b) After ingestion, oxygen-dependent microbial killing with phagolysosomes is initiated. Membrane-bound NADPH-oxidase vesicles fuse with the phagosome, creating high levels of reactive oxygen species in phagolysosomes, forming superoxide $O_2^-$, which is then readily converted into hydrogen peroxide by superoxide dismutase, and enhanced by the presence of myeloperoxidase converting hydrogen peroxide into hydrochloric acid. (c) Following phagocytosis, oxygen-independent digestive enzymes are trafficked in vesicles which also fuse with the phagolysosome, a process called intracellular degranulation. The enzyme names need not be committed to memory.

Phagocytes have both **aerobic** and **anaerobic** mechanisms for destroying what they eat. Aerobic destruction requires oxygen, and is a process known as the **respiratory burst**. The molecular pathways and how they malfunction are discussed in #15: *Immunodeficiency*. To give you an overview here, the respiratory burst first creates an oxygen-free radical ($O^*$), then forms hydrogen peroxide ($H_2O_2$), then bleach (HOCl). **Catalase-negative organisms** are vulnerable to $O^*$, $H_2O_2$, and HOCl—everything. **Catalase-positive** organisms (aka staph) protect themselves from $O^*$ and $H_2O_2$, so they require the HOCl step to work. **Encapsulated organisms** defend against phagocytosis. Once engulfed into a phagosome, the pathogen will be fused and exposed to the lysosome. Bacterial survival is therefore dependent mostly on evasion of initial phagocytosis.